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Supplemental Information

Polysaccharides from *Ganoderma lucidum* Promote Cognitive Function and Neural Progenitor Proliferation in Mouse Model of Alzheimer's Disease

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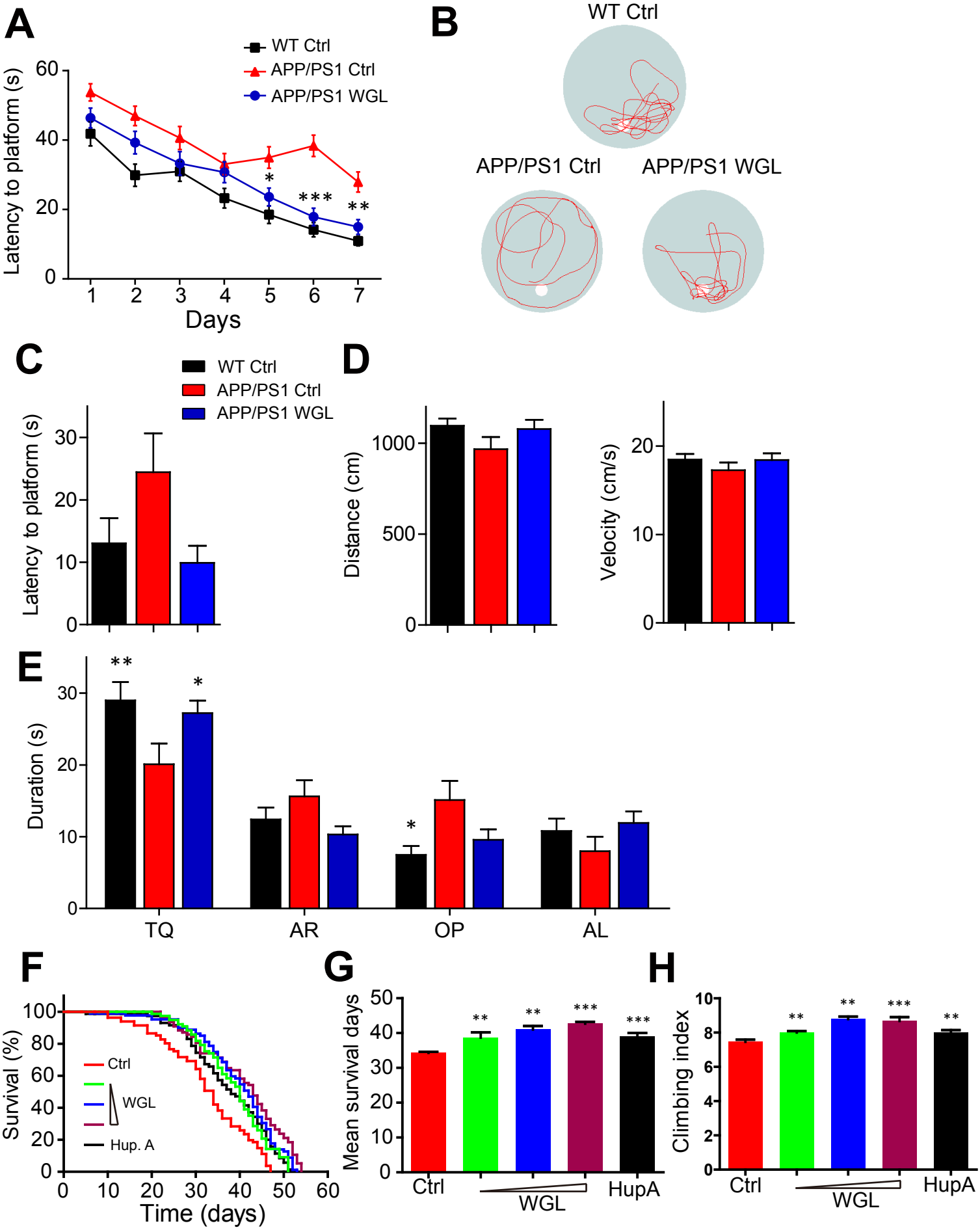
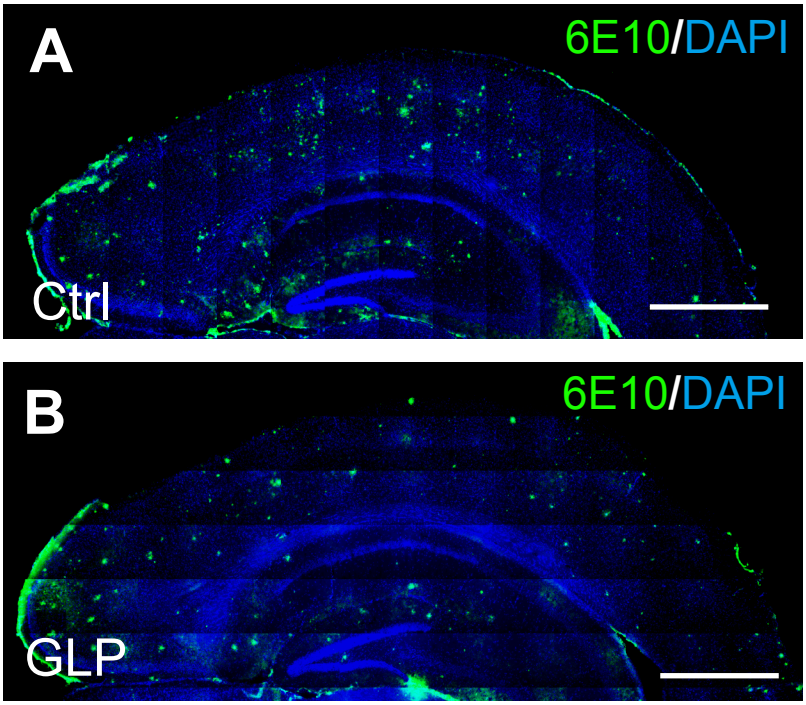
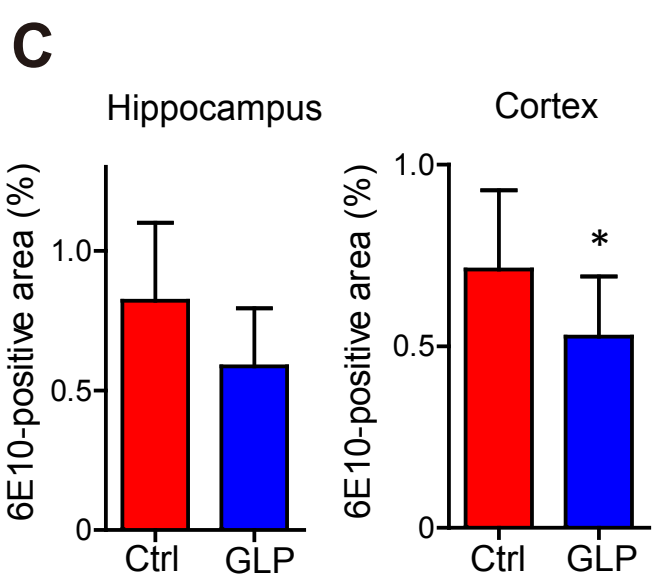


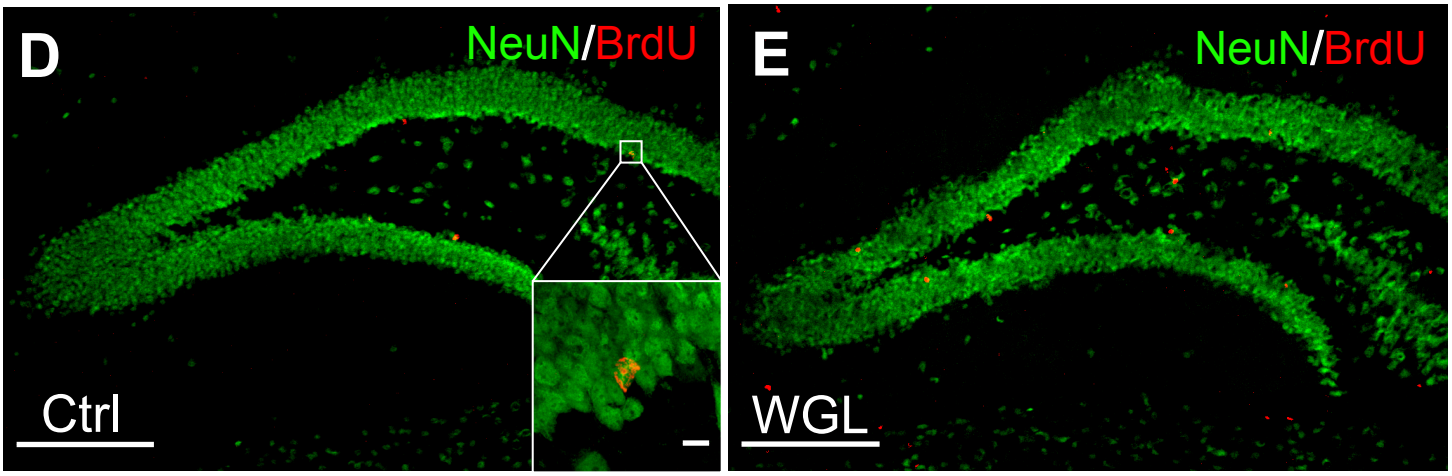
Figure S1 (Related to Figure 1): The water extract of Ganoderma lucidum (WGL) improves cognitive function in APP/PS1 mice and ameliorates AD associated defects in Aβ transgenic Drosophila.

(A) MWM test for WGL and vehicle (Ctrl)-treated APP/PS1 and wild type (WT) mice (n = 12-13 per group). The mean escape latency was given for different test days. (B) Representative mice search paths from different groups in probe trial of MWM on day 7. (C) The latency to platform in probe trial for each group of mice (n = 12-13 per group). (D) The swimming distance and velocity in the probe trial were shown (n = 12-13 per group). (E) The time spent by mice in the target quadrant (n = 12-13 per group). TQ: Target quadrant; AR: Adjacent right; OP: Opposite; AL: Adjacent left. (F, G) Aβ transgenic

flies were cultured on food containing different concentrations of WGL (the triangle symbol stands for concentrations from low to high: 3, 10, 30 and 100 μg/ml) or Huperzine A (2 μM). (F) Survival curves for flies treated with either WGL or Huperzine A were shown. (G) The mean survival days were calculated according to the survival curves. (H) The climbing ability of Aβ transgenic flies flies treated with WGL or Huperzine A at day 20. Quantifications are presented as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001, analyzed by two-way ANOVA test (A, E) followed by Bonferroni test, or one-way ANOVA test (C, D, G, H) followed by Bonferroni test. Survival curves were plotted using Kaplan–Meier survival analysis. The data are presented as mean ± SEM; each value in (G, H) represents the mean of three experiments. HupA= Huperzine A.







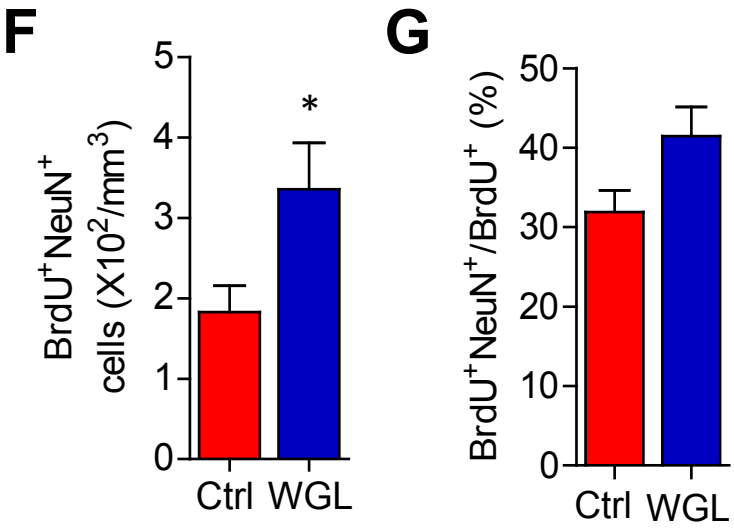


Figure S2 (Related to Figure 2): Effect of GLP and WGL on amyloid deposits or neurogenesis in transgenic AD mice.
(A, B) 6E10 (green) and DAPI (blue) staining of brain sections from AD mice treated with vehicle (Ctrl, A) and GLP (B). (C) Quantification of $6E10^+$ areas from sections as in (A, B). n = 9-10 per group. (D,E) BrdU (red) and NeuN (green) staining of DG sections from mice treated with vehicle (Ctrl, D) and WGL (E). (F) Quantification of $BrdU^+NeuN^+$ cells from sections as in (D, E). n = 9-12 per group. (G) Proportion of $BrdU^+NeuN^+$ cells in $BrdU^+$ cells (n = 9-12 per group). Quantifications are presented as mean ± SEM; *P < 0.05, analyzed by two-tailed t test (C, F, G) compared with APP/PS1 Ctrl group; scale bars in (A, B), 1000 μm; scale bars in (D, E), 100 μm; Insets were images of high magnification with scale bars of 10 μm.

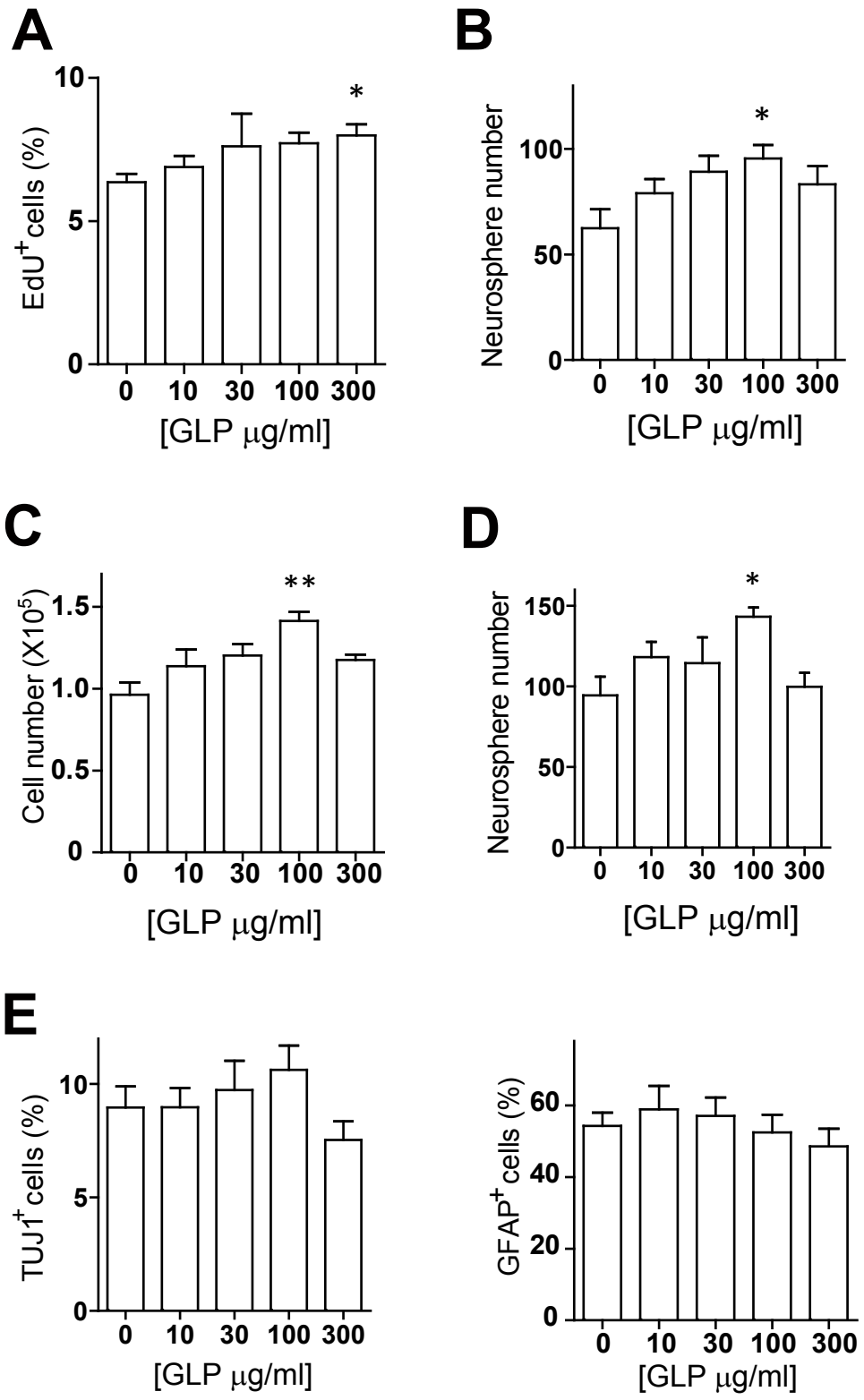


Figure S3 (Related to Figure 4): Effect of Ganoderma lucidum polysaccharides (GLP) on embryonic NPC proliferation and adult NPC differentiation.
(A) Monolayer embryonic neural precursor cultures were treated with GLP of different concentrations for 24 hours in the culture medium containing 1 ng/ml EGF and 1 ng/ml bFGF. EdU was added 2 hours prior to fixation. The percentage of EdU+ cells among total cells in the culture was determined. n = 4 independent experiments.
(B-D) Embryonic neural precursors were cultured in neurosphere-forming conditions in the presence of absence of GLP. 6 days later, the number of (B) neurospheres and (C) cells were quantified for each condition. All neurospheres from each condition were collected,

dissociated and re-plated in the same culture condition. (D) 6 days later, the number of neurospheres was determined. n = 4 independent experiments. (E) Percentage of TUJ1+ (left) or GFAP+ (right) cells in the adult hippocampal NPC cultures treated with GLP of different concentrations after 5 days under the differentiation condition. Quantifications are presented as mean ± SEM of three independent experiments and are analyzed by one-way ANOVA followed by Bonferroni test.

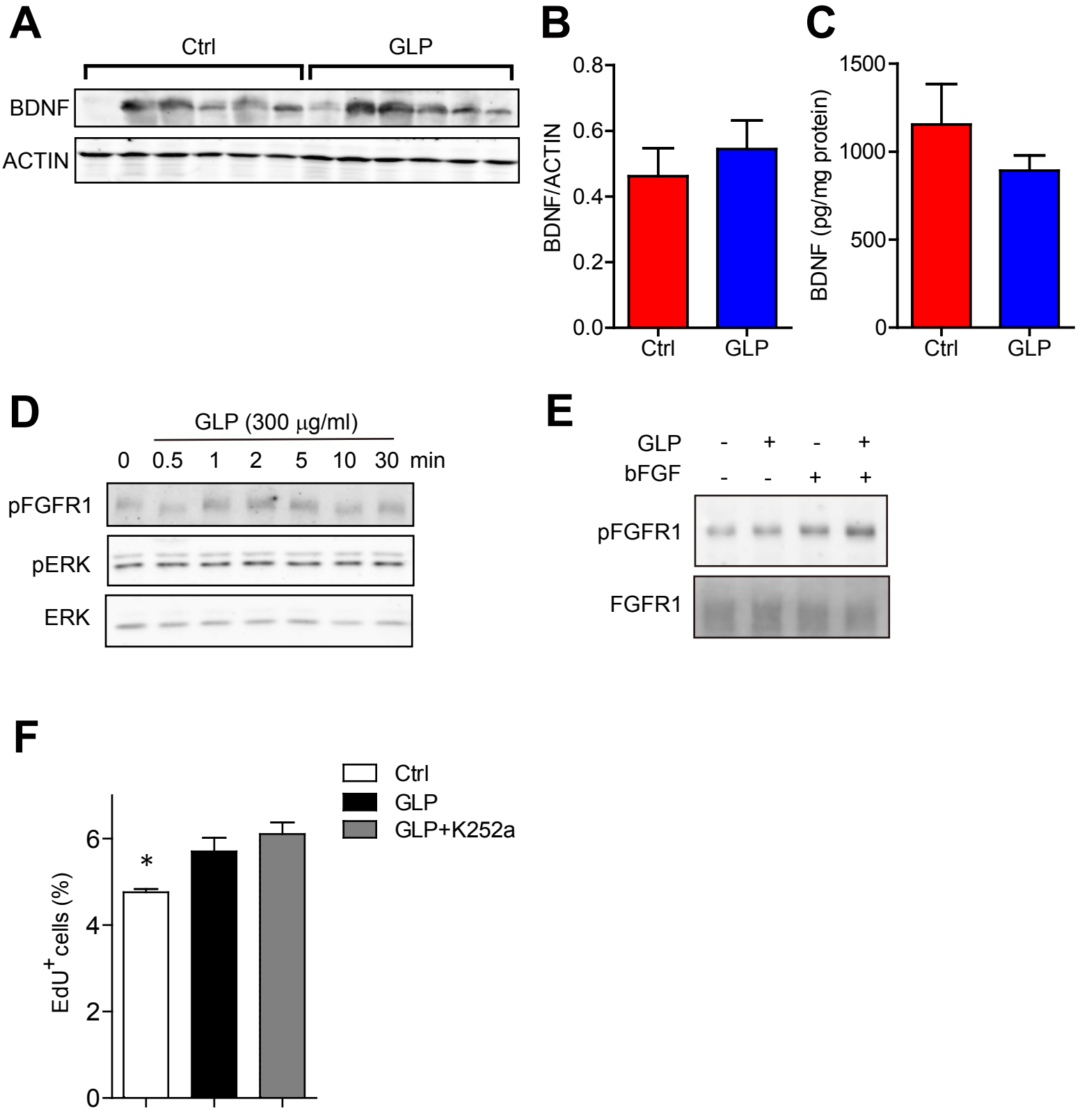


Figure S4 (Related to Figure 5): Effects of Ganoderma lucidum polysaccharides (GLP) BDNF signaling and FGFR signaling. (A, B) Western blot analysis of BDNF expression in brain tissues from transgenic AD mice treated with vehicle or GLP (n=6 for each group). Blots were probed for ACTIN as loading controls. (C) ELISA analysis of BDNF expression in brain tissues from transgenic AD mice treated with vehicle or GLP (n=6 for each group). Total protein was used for normalization. (D) Monolayer embryonic neural precursor culture was starved in the no-growth-factor medium for 3 hours. NPCs were then treated with 300 μg/ml GLP for the indicated time. Protein samples in the same experiment were analyzed on Western blots using antibodies against the phosphorylated FGFR1 and ERK. Blots were probed for total ERK as loading controls. Three independent experiments showed similar results. (E) NPCs were starved as in D, then they were treated with GLP 300 μg/ml, bFGF 0.5 ng/ml or their combination for 2 minutes. Protein samples were analyzed on Western blots using antibodies against the phosphorylated FGFR1 and ERK. Blots were probed for total FGFR1 and ERK as loading controls. Three independent experiments showed similar results. (F) NPC were pre-treated with TRK inhibitor K252a (200nM) for 30 minutes. 30 μg/ml GLP

were then added and the cells were incubated for additional 24 hrs. EdU incorporation on the last 2 hrs was visualized with staining and quantified. All the groups were compared with the cell culture treated with GLP alone. n = 3 independent experiments. Quantifications are presented as mean ± SEM; *P < 0.05, analyzed by two-tailed t test (B, C); or by one-way ANOVA followed by Fisher's protected least significant difference test (F).

Supplemental Experimental Procedures

Drosophila culture and stocks

Flies were raised at 25 ℃ on standard cornmeal-molasses-agar medium with 65% humidity and 12 h light/12 h dark cycle. The pan-neuronal elav-GAL4 was used to express transgenes as described (Brand and Perrimon, 1993). The upstream activating sequence (UAS) transgenic lines of Aβ42 were provided by Dr. FD Huang and have been described in detail (Zhao et al., 2010). Canton S (C.S) flies were used for wild type controls.

Drug preparation and treatments

The drugs were prepared with instant food, 0.5 g of Nestle Infant Cereal and 0.05% methyl p-hydroxybenzoate (Sinopharm) in a total of 1.4 ml of sterile distilled water. WGL were prepared to final concentration of 3, 10, 30 and 100 μg/ml. Huperzine A was diluted to a final concentration of 2 μM. Drug food was changed every 3 days.

Survival assay

Flies were collected within 24h after eclosion and were raised in 3 vials with 20 flies per vial. The number of dead flies was recorded every day. When all the flies were either dead or lost, the survival rate was analyzed using Kaplan–Meier survival statistic. The survival time was defined as median survival time (the days of 50% of Drosophila died in the group) ± SEM.

Locomotor assay

The locomotor assay was performed as described before (White et al., 2010). We transferred 10 male flies (n = 30 for each group) into a plastic 25 mL vial and placed them under red light. After a 30-min recovering phase, flies were gently tapped to the bottom of the vial. The climbing behavior was recorded with a video camera. After 10s of climbing, the number of flies between the 0, 5, 10, 15, 20 and 25 mL scale marks was recorded. The results for each group of flies were calculated by the formula below:

Climbing Index = (flies above 20 mL scale mark) ×1+ (flies between 15 and 20 mL scale marks) ×0.8+ (flies between 10 and 15 mL scale marks) ×0.6+ (flies between 5 and 10 mL scale marks) ×0.4 + (flies below 5 mL scale mark)×0.2.

In vitro neurosphere formation assay for embryonic mouse neural precursors

Embryonic neural precursors were seeded at density of 1.5 cell/μl in B27-supplemented DMEM/F-12

medium containing 10 ng/ml EGF and 10 ng/ml bFGF. NPCs were treated with different concentration of GLP for 6 days. Neurospheres with a diameter ≥50 μm were quantified, dissociated and re-plated at density of 1.5 cells/μl in the same untreated culture medium. The number of neurospheres was quantified 6 days later.

***In vitro* NPC differentiation**

Adult hippocampal NPCs were seeded at a density of 2.5 x10^4 cells/cm^2 onto PDL/laminin-coated 96-well plates in NeuroCult NSC proliferation medium containing 20 ng/ml bFGF. On the next day, the medium was replaced with NeuroCult NSC basal medium supplemented with differentiation supplement (StemCell Technologies) in the presence or absence of GLP. The medium was changed every 2 days. 5 days later, expression of the neuron marker TUJ1 and the astrocyte marker GFAP was detected.

Tissue processing

Left cerebral hemispheres from APP/PS1 mice treated with vehicle or GLP were isolated and stored at -80 ℃. To prepare tissue homogenate for western and ELISA, the frozen hemibrains were homogenized in homogenization buffer containing 250 mM sucrose, 20 mM Tris base, 1 mM EDTA, 1 mM EGTA, 1mM AEBSF, 1 mM aprotinin, 1 mM pepstatin, 1 mM leupeptin, and 1 mM Na_3PO_4. The protein concentration was determine using Pierce™ BCA Protein Assay (Thermo Fisher Scientific).

ELISA

Brain-derived neurotrophic factor (BDNF) protein in brain homogenate was measured with a commercially available mouse BDNF ELISA kit (Elabscience) following the manufacturer's protocol. The BDNF protein concentration was normalized with quantity of total protein.

Supplemental References

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White, K.E., Humphrey, D.M., and Hirth, F. (2010). The dopaminergic system in the aging brain of Drosophila. Front Neurosci 4, 205.

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